REMARKS

Status of the Claims

Pending claims

Claims 17 to 43 are pending.

Claims amended and added in the instant amendment

In the present response, claims 17 to 43 are amended, new claims 44 and 47 are added. Accordingly, after entry of the instant amendment, claims 17 to 47 are pending and under examination.

Outstanding Rejections

Claims 17 to 43 are rejected under 35 U.S.C. §112, first and second paragraphs. Applicants respectfully traverse all outstanding objections to the specification and rejection of the claims.

Support for the Claim Amendments

Support for the claim amendments can be found throughout the specification. Accordingly, Applicants respectfully submit that no new matter is introduced by the instant amendments.

Written Description issues

Claims 17 to 43 are rejected under 35 U.S.C. §112, first paragraph, as allegedly not containing subject matter which was not described in the specification in such a way as to reasonable convey to one skilled in the art that the inventors at the time the application was filed had possession of the invention.

BLAST programs

The Patent Office alleges that there is no support for the term "BLASTP" program in claims 17 and 18. The instant amendment addresses this issue.

80% sequence identity

The Patent Office alleges that there is no support for "80% sequence identity" at the amino acid level in claims 19 and 23. The instant amendment addresses this issue. The

claim is now directed to 80% sequence identity to a nucleic acid encoding a transaminase or aminotransferase activity.

The phrase "about 400 μmoles of α-keto acid per minute per mg of the enzyme"

The Patent Office alleges that there is no support for an enzyme that converts "about" 400 μmoles of α-keto acid per minute per mg of the enzyme. The instant amendment addresses this issue.

Specific transaminase/ aminotransferase functions

The Patent Office also alleges that it is unpredictable what will be the specific transaminase/ aminotransferase function of a protein having an amino acid sequence that is at least 70% homologous to an exemplary sequence of the invention.

Regarding claim 17, the instant amendment addresses this issue. After entry of the instant amendment, claim 17 is drawn to proteins having amino acid sequences that are at least 70% homologous to: SEQ ID NO:25 and having an aspartate transaminase activity, SEQ ID NO:26 and having an aspartate transaminase activity, SEQ ID NO:27 and having an adenosyl-8-amino-7-oxononanoate aminotransferase activity, SEQ ID NO:28 and having an acetylornithine aminotransferase activity, SEQ ID NO:29 and having an aspartate aminotransferase activity, SEQ ID NO:30 and having a glucosamine:fructose-6-phosphate aminotransferase activity, SEQ ID NO:31 and having a histidinol-phosphate aminotransferase activity, or, SEQ ID NO:32 and having a branched chain aminotransferase activity.

Regarding claims directed to isolated or recombinant polypeptides having transaminase or aminotransferase activity and having at least 70% sequence identity to sequence as set forth in SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 or SEQ ID NO:32 (see, e.g., new claim 49), Applicants respectfully submit that the claimed invention is sufficiently described in the specification such that one of ordinary skill in the art would have been able to ascertain the scope of the claims with reasonable clarity and recognize that Applicants' were in possession of the claimed invention at the time of filing. Applicants respectfully submit that because these claims were enabled by the specification (see discussion below) and because a representative number of species of the genus of functions was explicitly disclosed (i.e., the transaminase or aminotransferase activity can

comprise an aspartate transaminase activity, an aspartate transaminase activity, an adenosyl-8-amino-7-oxononanoate aminotransferase activity, an acetylornithine aminotransferase activity, an aspartate aminotransferase activity, a glucosamine:fructose-6-phosphate aminotransferase activity, a histidinol-phosphate aminotransferase activity, or a branched chain aminotransferase activity), the claims meet the written description requirements of section 112, first paragraph.

A single species is disclosed

The Patent Office also alleged that disclosing only a single species within the genus transaminase/ aminotransferase of any one of SEQ ID NOS:25 to 32 is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus.

Applicants respectfully submit that the claimed invention is sufficiently described in the specification so that one of ordinary skill in the art would be able to ascertain the scope of the claims with reasonable clarity and recognize that Applicants' were in possession of the claimed invention at the time of filing. Applicants respectfully submit that describing a genus of polypeptide or polynucleotides in terms of physico-chemical properties (e.g., a % sequence identity) as compared to an exemplary species (e.g., SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 or SEQ ID NO:32) and function (e.g., encoding a polypeptide having transaminase/ aminotransferase, and, in particular, SEQ ID NO:25 and having an aspartate transaminase activity, SEQ ID NO:26 and having an aspartate transaminase activity, SEQ ID NO:27 and having an adenosyl-8-amino-7-oxononanoate aminotransferase activity, SEQ ID NO:28 and having an acetylornithine aminotransferase activity, SEQ ID NO:29 and having an aspartate aminotransferase activity, SEQ ID NO:30 and having a glucosamine:fructose-6-phosphate aminotransferase activity, SEQ ID NO:31 and having a histidinol-phosphate aminotransferase activity, or, SEQ ID NO:32 and having a branched chain aminotransferase activity) satisfies the written description requirement of section 112, first paragraph.

Applicants respectfully aver that there is no bright line rule that a single species is insufficient to put one of skill in the art in possession of the attributes and features of all species with a genus. In fact, both the Patent Office and the Federal Circuit set forth conditions where a

single species is sufficient to put one of skill in the art in possession of the attributes and features of all species within a genus, where the genus is defined in terms of shared physical and structural properties with the single species.

Applicants respectfully refer to the USPTO guidelines concerning compliance with the written description requirement of U.S.C. §112, first paragraph, and note that the guidelines state that a description of a genus of polynucleotides in terms of its physico-chemical properties, e.g., a % sequence identity, to a single exemplary species, and a common function satisfies the written description requirement of section 112, first paragraph, for the genus of polynucleotides.

In Example 14 of the Guidelines (a copy of which is attached as Exhibit A), a claim reciting variants claimed by sequence identity to a sequence is sought (specifically, "A protein having SEQ ID NO:3 and variants thereof that are at least 95% identical to SEQ ID NO:3 and catalyze the reaction of A → B). In the example, the specification is described as providing SEQ ID NO:3 and a function for the protein. The specification contemplates, but does not exemplify variants of SEQ ID NO:3 that can have substitutions, deletions, insertions and additions. Procedures for making proteins with substitutions, deletions, insertions, and additions are routine in the art and an assay is described which will identify other proteins having the claimed catalytic activity. The analysis of Example 14 states that procedures for making variants (which have 95% sequence identity) are conventional in the art. The Guidelines conclusion states that the disclosure meets the requirements of 35 U.S.C. §112, first paragraph, as providing adequate written description for the claimed invention.

Analogously, the genus of peptides and polypeptides of the invention is described by structure (the exemplary SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 or SEQ ID NO:32), a physico-chemical property (percent sequence identify) and function (encoding a polypeptide having transaminase/ aminotransferase, and, in particular, SEQ ID NO:25 and having an aspartate transaminase activity, SEQ ID NO:26 and having an aspartate transaminase activity, SEQ ID NO:27 and having an adenosyl-8-amino-7-oxononanoate aminotransferase activity, SEQ ID NO:28 and having an acetylornithine aminotransferase activity, SEQ ID NO:29 and having an aspartate aminotransferase activity, SEQ ID NO:30 and having a glucosamine:fructose-6-phosphate

aminotransferase activity, SEQ ID NO:31 and having a histidinol-phosphate aminotransferase activity, or, SEQ ID NO:32 and having a branched chain aminotransferase activity). All species of the genus used in the claimed methods must have at least 70% or more sequence identity to a sequence as set forth in SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 or SEQ ID NO:32. The USPTO guidelines recognize that written description is met for a genus of polypeptides described by structure, a physico-chemical property (e.g., a % sequence identity) and a defined function, the genus of claimed polypeptides also meet the written description requirements of section 112.

The genus of nucleic acids of the invention also fully comply with the requirements for written description of a genus of nucleic acids as set forth in University of California v. Eli Lilly & Co., 43 USPQ2d 1398 (Fed. Cir. 1997). In Lilly, the Court stated that, "[a] description of a genus of cDNA may be achieved by means of a recitation of a representative number of cDNAs....*or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus.*" (emphasis added) Lilly, 43USPQ2d at 1406.

As noted above, the instant claims clearly set forth specific structural and physical characteristics of the claimed genus of transaminases/ aminotransferases. The claimed genus of polypeptides all must have transaminase/ aminotransferase activity and a specific physical characteristic, e.g., a % sequence identity to an exemplary nucleic acid. Therefore, the genus of claimed transaminases/ aminotransferases is defined via shared physical and structural properties in terms that "convey with reasonable clarity to those skilled in the art that Applicant, as of filing date sought, was in possession of invention." (Vas-Cath Inc. V. Mahukar, 19 USPQ2d 1111, (Fed Cir. 1991)).

More recently, the Federal Circuit stated

Similarly, in this court's most recent pronouncement, it noted:

> More recently, in Enzo Biochem, we clarified that Eli Lilly did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure.

Amgen, 314 F.3d at 1332 [Amgen Inc. v. Hoechst Marion Roussel Inc., 314 F.3d 1313, 1330, 65 USPQ2d 1385, 1397 (Fed. Cir. 2003)].

Moba, B.V. v. Diamond Automation, Inc., 2003 U.S. App. LEXIS 6285; Fed. Cir. 01-1063, -1083, April 1, 2003.

Analogously, the function of the claimed transaminases/ aminotransferases is sufficiently correlated to a particular, known structure (the exemplary sequences) and a physical (physico-chemical) property (percent sequence identity). Accordingly, the sequences of the invention are defined via shared physical and structural properties in terms that convey with reasonable clarity to those skilled in the art that Applicants, as of the filing date and at the time of the invention, were in possession of the claimed invention.

Applicants also respectfully refer to recently issued claims directed to genuses of sequences based on sequence identity (and stringent hybridization) to an exemplary specie, see, e.g., recently issued claims directed to, e.g., 72.5% sequence identity, as in USPN 6,593,514; 75% sequence identity, as in USPN 6,586,215; 80% sequence identity, as in USPN 6,596,926; 85% sequence identity, as in USPN 6,590,141 and USPN 6,586,179; 86% sequence identity, as in USPN 6,583,337; 90% sequence identity (and "stringent hybridization"), as in USPN 6,541,684 (see Exhibit B).

Accordingly, Applicants respectfully submit that the pending claims meet the written description and enablement requirements under 35 U.S.C. §112, first paragraph. In light of the above remarks, Applicants respectfully submit that amended claims are fully enabled by and described in the specification to overcome the rejection based upon 35 U.S.C. §112, first paragraph.

Enablement

Claims 17 to 43 are rejected under 35 U.S.C. §112, first paragraph, because the specification allegedly does not reasonably provide enablement for the claimed invention.

The Patent Office notes that the specification is enabling for a specific transaminase/ aminotransferase having an amino acid sequence as set forth in any one of the exemplary SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31 or SEQ ID NO:32.

However, it is alleged that the specification is not enabling for transaminases/ aminotransferases having undefined specificity.

With respect to claim 17, Applicants respectfully aver that this issue is addressed in the instant amendment. After entry of the amendment, claim 17 is drawn to proteins having amino acid sequences that are at least 70% homologous to: SEQ ID NO:25 and having an aspartate transaminase activity, SEQ ID NO:26 and having an aspartate transaminase activity, SEQ ID NO:27 and having an adenosyl-8-amino-7-oxononanoate aminotransferase activity, SEQ ID NO:28 and having an acetylornithine aminotransferase activity, SEQ ID NO:29 and having an aspartate aminotransferase activity, SEQ ID NO:30 and having a glucosamine:fructose-6-phosphate aminotransferase activity, SEQ ID NO:31 and having a histidinol-phosphate aminotransferase activity, or, SEQ ID NO:32 and having a branched chain aminotransferase activity.

Applicants respectfully maintain that the specification enabled the skilled artisan at the time of the invention to identify, and make and use, the claimed genus of transaminases/ aminotransferases. As declared by Dr. Jay Short (see attached Rule 132 declaration), the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art, e.g., in identifying and screening polypeptides, and nucleic acids encoding these polypeptides, for transaminase or aminotransferase activities, was very high. As declared by Dr. Short, using the teaching of the specification, one skilled in the art only needed routine screening procedures to determine if a polypeptide had transaminase or aminotransferase activity. Dr. Short states that one skilled in the art only needed routine screening procedures to screen for a specific type of transaminase or aminotransferase activity, e.g., an aspartate transaminase activity, an aspartate transaminase activity, an adenosyl-8-amino-7-oxononanoate aminotransferase activity, an acetylornithine aminotransferase activity, an aspartate aminotransferase activity, a glucosamine:fructose-6-phosphate aminotransferase activity, a histidinol-phosphate aminotransferase activity, a branched chain aminotransferase activity or any other category of transaminase or aminotransferase activity. Accordingly, it would not have taken undue experimentation for the skilled artisan at the time of the invention to determine the specific transaminase or aminotransferase activity of any protein.

It is also alleged that the specification does not enable the broad scope of structures (e.g., modifications and fragments) encompassed by claims drawn to at least 70% sequence identity to an exemplary sequence because, inter alia, it is not routine in the art to screen for multiple substitutions and multiple modifications.

Applicants respectfully maintain that the specification enabled the skilled artisan at the time of the invention to identify, and make and use, the claimed genus of transaminases/ aminotransferases. As declared by Dr. Jay Short, the state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art, e.g., in screening enzymes, and nucleic acids encoding enzymes, for transaminase/ aminotransferase activity, was very high.

As declared by Dr. Short, using the teaching of the specification, one skilled in the art could have selected routine methods known in the art at the time of the invention to express variants and fragments of nucleic acids encoding any one of the exemplary enzymes of the invention and screen them for expression of polypeptides and peptides having a transaminase/ aminotransferase activity. Dr. Short declares that one skilled in the art could have used routine protocols known in the art at the time of the invention, including those described in the instant specification, to make and screen for polypeptides and peptides having transaminase/ aminotransferase activity and a percent sequence identity to an exemplary sequence of the invention, and nucleic acids encoding them.

As declared by Dr. Short, while the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results (e.g., making and successfully screening for variant nucleic acids or fragments encoding polypeptides and peptides having transaminase/ aminotransferase activity or making and successfully screening for polypeptides and peptides having transaminase/ aminotransferase activity) predictable.

Dr. Short declares that it would not have required any knowledge or guidance as to which are the specific structural elements, e.g., amino acid residues, that correlate with a transaminase/ aminotransferase activity to create variants or fragments of exemplary nucleic acids and test them for the expression of polypeptides or peptides having transaminase/ aminotransferase activity. Furthermore, Dr. Short declares that at the time of the invention success was predictable and protocols were routine to make and screen for multiple substitutions

and multiple modifications of polypeptides or peptides having transaminase/ aminotransferase activity.

Accordingly, it would not have taken undue experimentation to make and use the claimed invention, including making and screening for a genus of polypeptides or peptides having transaminase/ aminotransferase activity with a specific sequence identity to an exemplary transaminase/ aminotransferase of the invention.

Issues under 35 U.S.C. §112, second paragraph

Claims 17 to 43 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite.

The phrase "an enzyme with activity"

The Patent Office has concerns with the phrase "an enzyme with activity". The instant amendment addresses this issue.

Specific activities of transaminases/ aminotransferases

The Patent Office alleges that some of the specific activities of the claimed transaminases/ aminotransferases are not defined in the specification or readily known in the art.

After entry of the instant amendment, claim 17 is drawn to proteins having amino acid sequences that are at least 70% homologous to: SEQ ID NO:25 and having an aspartate transaminase activity, SEQ ID NO:26 and having an aspartate transaminase activity, SEQ ID NO:27 and having an adenosyl-8-amino-7-oxononanoate aminotransferase activity, SEQ ID NO:28 and having an acetylornithine aminotransferase activity, SEQ ID NO:29 and having an aspartate aminotransferase activity, SEQ ID NO:30 and having a glucosamine:fructose-6-phosphate aminotransferase activity, SEQ ID NO:31 and having a histidinol-phosphate aminotransferase activity, or, SEQ ID NO:32 and having a branched chain aminotransferase activity.

Applicants respectfully aver that aminotransferase and transaminase activities, including aspartate transaminase activity, adenosyl-8-amino-7-oxononanoate aminotransferase activity, acetylornithine aminotransferase activity, glucosamine:fructose-6-phosphate aminotransferase activity, histidinol-phosphate aminotransferase activity and branched chain aminotransferase activity, were well known in the art at the time of the invention. Dr. Short, an

expert in the field of molecular biology and enzyme development at the time of the invention has declared that aminotransferase and transaminase activities, including aspartate transaminase activity, adenosyl-8-amino-7-oxononanoate aminotransferase activity, acetylornithine aminotransferase activity, glucosamine:fructose-6-phosphate aminotransferase activity, histidinol-phosphate aminotransferase activity, and branched chain aminotransferase activity, were all well known in the art at the time of the invention. Accordingly, it was not necessary to include specifics about aminotransferase or transaminase activities, including aspartate transaminase activity, adenosyl-8-amino-7-oxononanoate aminotransferase activity, acetylornithine aminotransferase activity, glucosamine:fructose-6-phosphate aminotransferase activity, histidinol-phosphate aminotransferase activity or branched chain aminotransferase activity into the specification to satisfy the requirements of section 112, second paragraph.

It is further alleged that it is not defined and is unclear what is the difference between the various aminotransferase or transaminase activities of SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:29, having aspartate transaminase activity, and SEQ ID NO:32, having a branched chain aminotransferase activity. However, as declared by Dr. Short, at the time of the invention aspartate transaminases and branched chain aminotransferases (and the other activities of polypeptides of the invention, including the explicitly recited activities) were well known in the art. Dr. Short declares that at the time of the invention the skilled artisan using routine screening methods would have been above to distinguish between a polypeptide having any aminotransferase or transaminase activity, including determining (distinguishing between) aspartate transaminase activity and a polypeptide having branched chain aminotransferase activity. For example, see, Coleman (1971) "Branched chain amino acid aminotransferase of Salmonella typhimurium. II. Kinetic comparison with the enzyme from Salmonella montevideo," J. Biol. Chem. 246(5):1310-1312; Taylor (1970) "Branched chain amino acid aminotransferase. IV. Kinetics of the transamination reactions," J. Biol. Chem. 245(19):4880-4885, discussing branched chain amino acid aminotransferases; and, Levy (1972) "Aspartate aminotransferase (SGOT) assay in serum," Clin. Chem. 18(12):1539-1540, and U.S. Patent No. 4,710,467, discussing aspartate aminotransferase activity.

CONCLUSION

In view of the foregoing amendment and remarks, it is believed that the Examiner can properly withdraw the rejection of the pending claims under 35 U.S.C. §112, first and second paragraphs. Applicants respectfully submit that all claims pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If necessary, please apply additional and necessary charges, and apply all credits, to Deposit Account No. 06-1050.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (858) 678-5070.

Respectfully submitted,

Date: 11.02.2003

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Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

Exhibit B

United States Patent **6,596,926**
Famodu , et al. **July 22, 2003**

Phosphatidylcholine biosynthetic enzymes

Abstract

This invention relates to an isolated nucleic acid fragment encoding phosphatidylethanolamine N-methyltransferase biosynthetic enzyme. The invention also relates to the construction of a chimeric gene encoding all or a portion of the phosphatidylethanolamine N-methyltransferase biosynthetic enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of phosphatidylethanolamine N-methyltransferase biosynthetic enzyme in a transformed host cell.

Inventors: **Famodu; Omolayo O.** (Newark, DE); **Kinney; Anthony J.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **668262**

Filed: **September 22, 2000**

Current U.S. Class: **800/281**; 435/6; 435/69.1; 435/183; 435/410; 435/419; 435/252.3; 435/320.1; 530/350; 530/370; 536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.33; 800/278; 800/295

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N 005/14; C12N 009/00

Field of Search: 435/6,69.1,183,410,419,252.3,320.1 530/350,370 536/23.2,23.6,24.1,24.3,24.33 800/278,295,281

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Primary Examiner: Bui; Phuong T.

Parent Case Text

This application claims the benefit of U.S. Provisional Application No. 60/155,626, filed Sep. 23, 1999.

Claims

What is claimed is:

1. An isolated ***polynucleotide*** comprising:

(a) a nucleotide sequence encoding a polypeptide having phosphatidylethanolamine N-methyltransferase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 80% sequence ***identity*** based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 85% sequence ***identity*** based on the Clustal alignment method.

3. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 90% sequence ***identity*** based on the Clustal alignment method.

4. The ***polynucleotide*** of claim 1 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:20 have at least 95% sequence ***identity*** based on the Clustal alignment method.

5. The ***polynucleotide*** of claim 1 wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:20.

6. The ***polynucleotide*** of claim 1 wherein the nucleotide sequence comprises the

nucleotide sequence of SEQ ID NO:19.

7. A vector comprising the ***polynucleotide*** of claim 1.

8. A recombinant DNA construct comprising the ***polynucleotide*** of claim 1 operably linked to a regulatory sequence.

9. A method for transforming a cell comprising transforming a cell with the ***polynucleotide*** of claim 1.

10. A cell comprising the recombinant DNA construct of claim 8.

11. A method for producing a plant comprising transforming a plant cell with the ***polynucleotide*** of claim 1 and regenerating a plant from the transformed plant cell.

12. A plant comprising the recombinant DNA construct of claim 8.

13. A seed comprising the recombinant DNA construct of claim 8.

United States Patent **6,593,514**
Cahoon , et al. **July 15, 2003**

Method for the production of calendic acid, a fatty acid containing delta-8,10,12 conjugated double bonds and related fatty acids having a modification at the delta-9 position

Abstract

The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with modification of the delta-9 position of fatty acids, in particular, formation of conjugated double bonds are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles. The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with formation of a trans delta-12 double bond also are disclosed. Chimeric genes incorporating such nucleic acid fragments and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles.

Inventors: **Cahoon; Edgar Benjamin** (Wilmington, DE); **Hitz; William Dean** (Wilmington, DE); **Ripp; Kevin G.** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

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Intern'l Class: A01H 005/00; C12N 015/82; C07H 021/04

Field of Search: 800/281,298 435/69.1,419 536/23.6

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Primary Examiner: McElwain; Elizabeth F.

Parent Case Text

This application claims priority benefit of U.S. Provisional Application No. 60/149,050 filed Aug. 16, 1999, now abandoned.

Claims

What is claimed is:

1. A chimeric gene comprising an isolated ***nucleic acid*** fragment encoding a plant fatty acid modifying enzyme associated with conjugated double bond formation comprising a delta-9 position of fatty acids having an amino acid ***identity*** of at least 72.5% based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:2 or 4

wherein said fragment or a functionally equivalent subfragment thereof or a complement thereof is operably linked to suitable regulatory sequences.

2. The chimeric gene of claim 1 wherein the ***nucleic acid*** fragment is isolated from Calendula officinalis.

3. The chimeric gene of claim 1 wherein the plant fatty acid modifying enzyme is associated with the formation of calendic acid.

4. A transformed host cell or plant comprising in its genome the chimeric gene of claim 1.

5. A transformed host cell or plant comprising in its genome the chimeric gene of claim 2.

6. A transformed host cell or plant comprising in its genome the chimeric gene of claim 3.

7. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:

(a) transforming a host cell or plant with the chimeric gene of claim 1;

(b) growing the transformed host cell or plant under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

8. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:

(a) transforming a host cell or plant with the chimeric gene of claim 2;

(b) growing the transformed host cell or plant under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

9. A method of altering the level of fatty acids in a host cell or plant wherein said fatty acids comprise a modification at a delta-9 position, said method comprising:

(a) transforming a host cell or plant with the chimeric gene of claim 3;

(b) growing the transformed host cell or plant under conditions suitable for the expression

of the chimeric gene; and

(c) selecting those transformed host cells or plants having altered levels of fatty acids comprising a modified delta-9 position.

10. The method of claim 7, 8, or 9 wherein the host cell or plant is selected from the group consisting of plant cells and microorganisms.

11. The method of claim 7, 8, or 9 and wherein the level of calendic acid is altered.

12. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 1;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

13. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 2;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

14. A method for producing fatty acid modifying enzymes associated with modification of a delta-9 position of fatty acids which comprises:

(a) transforming a microbial host cell with the chimeric gene of claim 3;

(b) growing the transformed host cell under conditions suitable for the expression of the chimeric gene; and

(c) selecting those transformed host cells containing altered levels of protein encoded by the chimeric gene.

15. The method of claim 12, 13, or 14 wherein the fatty acid modifying enzyme is associated with the formation of calendic acid or dimorphecolic acid.

United States Patent **6,590,141**
Frohberg **July 8, 2003**

Nucleic acid molecules from plants encoding enzymes which participate in starch synthesis

Abstract

Nucleic acid molecules are described which encode enzymes which participate in starch synthesis in plants. These enzymes are a new isoform of starch synthase. There are furthermore described vectors for generating transgenic plant cells and plants which synthesize a modified starch. There are furthermore described methods for the generation of these transgenic plant cells and plants, and methods for producing modified starches.

Inventors: **Frohberg; Claus** (Berlin, DE)
Assignee: **Aventis CropScience GmbH** (Frankfurt, DE)
Appl. No.: **638524**
Filed: **August 11, 2000**

Foreign Application Priority Data

Aug 11, 1999[DE] 199 37 348

Current U.S. Class: **800/284**; 800/278; 800/286; 800/320.1; 435/69.1; 435/101; 435/320.1; 435/419; 435/468; 536/23.6

Intern'l Class: C12N 015/29; C12N 015/82; C12N 005/04; A01H 005/00; C12P 019/04

Field of Search: 536/23.6 435/69.1,468,320.1,419,101 800/278,284,320.1,286

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Primary Examiner: Fox; David T.
Attorney, Agent or Firm: Frommer Lawrence & Haug LLP

Claims

I claim:

1. An isolated ***nucleic acid*** molecule encoding a protein with the bioactivity of a starch synthase selected from the group consisting of

(a) ***nucleic acid*** molecules which encode a protein with the amino acid sequence indicated under SEQ ID No. 2;

(b) ***nucleic acid*** molecules which encompass the nucleotide sequence shown under SEQ ID No. 1 or a complementary sequence thereof;

(c) ***nucleic acid*** molecules which encompass the coding region of the nucleotide sequence of the cDNA present in plasmid IR 65/87 (deposit number DSM 12970) or a complementary sequence thereof;

(d) ***nucleic acid*** molecules whose nucleotide sequence deviates from the sequence of the ***nucleic acid*** molecules mentioned under (a), (b) or (c) owing to the degeneracy of the genetic code;

(e) ***nucleic acid*** molecules which have over 85% sequence ***identity*** with SEQ ID NO:1; and

(f) ***nucleic acid*** molecules which constitute allelic variants of the nucleic acid molecules indicated under (a), (b), (c), (d) or (e).

2. The ***nucleic acid*** molecule as claimed in claim 1 which is a DNA molecule.

3. The ***nucleic acid*** molecule as claimed in claim 1 which is an RNA molecule.

4. A vector comprising a ***nucleic acid*** molecule as claimed in claim 1.

5. The vector as claimed in claim 4 comprising one or more regulatory elements which ensure the transcription of said ***nucleic acid*** molecules and/or the synthesis of a translatable RNA in a pro- and/or eukaryotic cell.

6. The vector as claimed in claim 4, wherein said ***nucleic acid*** molecule is linked in sense orientation to regulatory elements which ensure the transcription and synthesis of a translatable RNA in pro- and/or eukaryotic cells, or wherein said ***nucleic acid*** molecule is linked in anti-sense orientation to regulatory elements which ensure the transcription and synthesis of a non-translatable RNA in pro- and/or eukaryotic cells.

7. A host cell which is transformed with a ***nucleic acid*** molecule as claimed in claim 1 or a vector as claimed in claim 4, or a cell which is derived from the host cell and which comprises the vector of claim 4.

8. The host cell as claimed in claim 7 which is a plant cell.

9. A method for producing a protein encoded by the ***nucleic acid*** molecule of claim 1, in which a host cell as claimed in claim 7 is cultured under conditions which permit the synthesis of the protein, and the protein is isolated from the cultured cells and/or the culture medium.

10. The plant cell of claim 8, wherein said ***nucleic acid*** molecule which encodes a protein with the bioactivity of a starch synthase is under the control of regulatory elements which permit the transcription of a translatable mRNA in plant cells.

11. The plant cell of claim 8, wherein the activity of a protein encoded by the ***nucleic acid*** molecule of claim 1 is increased in this plant cell compared with corresponding, non-genetically-modified plant cells from wild-type plants.

12. A plant comprising plant cells as claimed in claim 8.

13. The plant as claimed in claim 12 which is a crop plant.

14. The plant as claimed in claim 12 which is a starch-storing plant.

15. The plant as claimed in claim 12 which is a maize plant.

16. A method for generating a transgenic plant cell, wherein a plant cell is subjected to genetic modification by introducing a ***nucleic acid*** molecule as claimed in claim 1 and/or a vector as claimed in claim 4.

17. A method for generating a transgenic plant, wherein

(a) a plant cell is subjected to genetic modification by introducing a ***nucleic acid*** molecule as claimed in claim 1 and/or a vector as claimed in claim 4; and

(b) a plant is regenerated from this cell; and, if appropriate,

(c) more plants are generated from the plant of (b).

18. Propagation material of a plant comprising plant cells as claimed in claim 8.

19. A method for producing a modified starch obtained from the host cell of claim 8, from the plant of claim 12, or from the propagation material of 18, comprising the step of extracting the starch from a plant cell as claimed in claim 8, from a plant as claimed in claim 12 and/or from propagation material as claimed in claim 18.

United States Patent **6,586,215**
Yaver , et al. **July 1, 2003**

Polypeptides having peroxidase activity and nucleic acids encoding same

Abstract

The present invention relates to isolated polypeptides having peroxidase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

Inventors: **Yaver; Debbie** (Davis, CA); **McArdle; Barbara** (Davis, CA)

Assignee: **Novozymes Biotech, Inc.** (Davis, CA)

Appl. No.: **885329**

Filed: **June 19, 2001**

Current U.S. Class: **435/192**; 435/6; 435/320.1; 435/325; 435/252.3; 536/23.1; 536/23.2

Intern'l Class: C12N 009/08; C12N 015/00; C12N 005/00; C12Q 001/68; C07H 021/04

Field of Search: 435/192,6,252.3,320.1 536/23.2,23.1

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Primary Examiner: Monshipouri; M.
Attorney, Agent or Firm: Stames; Robert L.

Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. application Ser. No. 09/596,824 filed Jun. 19, 2000 now U.S. Pat. No. 6,372,464 issued Apr. 16, 2002, which application is fully incorporated herein by reference.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** sequence encoding a polypeptide having peroxidase activity, selected from the group consisting of:

(a) a ***nucleic acid*** sequence encoding a polypeptide having an amino acid sequence which has at least 75% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6, or at least 85% ***identity*** with amino acids 22 to 385 of SEQ ID NO:4;

(b) a ***nucleic acid*** sequence encoding a polypeptide having an amino acid sequence which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5, or at least 85% homology with nucleotides 2008 to 3462 of SEQ ID NO:3;

(c) a ***nucleic acid*** sequence which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) the cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii); and

(d) a fragment of (a), (b), or (c), which encodes a polypeptide having peroxidase activity.

2. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide having an amino acid sequence which has at least 75% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

3. The ***nucleic acid*** sequence of claim 2, which encodes a polypeptide having an amino acid sequence which has at least 80% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

4. The ***nucleic acid*** sequence of claim 3, which encodes a polypeptide of having an amino acid sequence which has at least 85% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

5. The ***nucleic acid*** sequence of claim 4, which encodes a polypeptide having an amino acid sequence which has at least 90% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6.

6. The ***nucleic acid*** sequence of claim 5, which encodes a polypeptide having an amino

acid sequence which has at least 95% ***identity*** with amino acids 22 to 370 of SEQ ID NO:2 or amino acids 19 to 362 of SEQ ID NO:6 .

7. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6 .

8. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, or a fragment thereof having peroxidase activity.

9. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide consisting of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO6.

10. The ***nucleic acid*** sequence of claim 1, which encodes a polypeptide which consists of amino acids 22 to 370 of SEQ ID NO:2, amino acids 22 to 365 of SEQ ID NO:4, or amino acids 19 to 362 of SEQ ID NO:6.

11. The ***nucleic acid*** sequence of claim 1, which has at least 75% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

12. The ***nucleic acid*** sequence of claim 11, which has at least 80% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

13. The ***nucleic acid*** sequence of claim 12, which has at least 85% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

14. The ***nucleic acid*** sequence of claim 13, which has at least 90% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

15. The ***nucleic acid*** sequence of claim 14, which has at least 95% homology with nucleotides 772 to 2302 of SEQ ID NO:1 or nucleotides 2848 to 4247 of SEQ ID NO:5.

16. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:1.

17. The ***nucleic acid*** sequence of claim 1, which has the ***nucleic acid*** sequence of nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5.

18. The ***nucleic acid*** sequence of claim 1, which hybridizes under high stringency conditions with (i) nucleotides 772 to 2302 of SEQ ID NO:1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2848 to 4247 of SEQ ID NO:5, (ii) me cDNA sequence contained in nucleotides 772 to 2302 of SEQ ID NO;1, nucleotides 2008 to 3462 of SEQ ID NO:3, or nucleotides 2845 to 4247 of SEQ ID NO:5, or (iii) a complementary strand of (i) or (ii).

19. The ***nucleic acid*** sequence of claim 1, which is contained in plasmid pBM37-7 which is contained in E. coli NRRL B-30280, plasmid pBM38-1 which is contained in E. coli NRRL B-30281, or plasmid pBM39-1 which is contained in E. coli NRRL B-30282.

20. A ***nucleic acid*** construct comprising the ***nucleic acid*** sequence of claim 1, operably linked to one or more control sequences which direct the production of the polypeptide in a suitable expression host.

21. A recombinant expression vector comprising the ***nucleic acid*** construct of claim 20, a promoter, and transcriptional and translational stop signals.

22. A recombinant host cell comprising the ***nucleic acid*** construct of claim 20.

23. A method for producing a polypeptide having peroxidase activity comprising

(a) cultivating the host cell of claim 22, under conditions suitable for production of the polypeptide; and

(b) recovering the polypeptide.

United States Patent **6,586,179**
Jegla , et al. **July 1, 2003**

Human Eag2

Abstract

The invention provides isolated nucleic acid and amino acid sequences of Eag2, antibodies to Eag2, methods of detecting Eag2, and methods of screening for modulators of Eag2 potassium channels using biologically active Eag2. The invention further provides, in a computer system, a method of screening for mutations of human Eag2 genes as well as a method for identifying a three-dimensional structure of Eag2 polypeptide monomers.

Inventors: **Jegla; Timothy J.** (Durham, NC); **Liu; Yi** (Cary, NC)
Assignee: **ICAgen, Incorporated** (Durham, NC)
Appl. No.: **614480**
Filed: **July 10, 2000**

Current U.S. Class:	**435/6**; 536/23.1; 435/69.1; 435/325; 435/320.1; 435/252.3; 530/350
Intern'l Class:	C12Q 001/68; C07H 017/00; C12P 021/06; C07K 014/00
Field of Search:	536/23.1 435/7.1,325,320.1,252.3 530/350 436/6

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Primary Examiner: Carlson; Karen Cochrane
Attorney, Agent or Firm: Townsend and Townsend and Crew LLP

Parent Case Text

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Ser. No. 60/143,467, filed Jul. 13, 1999, herein incorporated by reference in its entirety.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and wherein said ***nucleic acid*** specifically hybridizes under stringent conditions to SEQ ID NO:1, wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

2. An isolated ***nucleic acid*** encoding a polypeptide comprising an alpha subunit of a potassium channel, wherein the subunit:

(i) forms, with at least one additional Eag family alpha subunit, a potassium channel having the characteristic of voltage sensitivity; and

(ii) comprises an amino acid sequence that has greater than 85% amino acid ***identity*** to the amino acid sequence of SEQ ID NO:2.

3. The isolated ***nucleic acid*** of claim 1, wherein the polypeptide specifically binds to polyclonal antibodies generated against SEQ ID NO:2.

4. The isolated ***nucleic acid*** of claim 1, wherein the ***nucleic acid*** encodes human Eag2.

5. The isolated acid of claim 1, wherein the ***nucleic acid*** encodes an amino acid sequence of SEQ ID NO:2.

6. The isolated ***nucleic acid*** sequence of claim 1, wherein the ***nucleic acid*** has a nucleotide sequence of SEQ ID NO:1.

7. The isolated ***nucleic acid*** of claim 1, wherein the ***nucleic acid*** is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as primers selected from the group consisting of:

ATGCCGGGGGGCAAGAGAGGGCTG (SEQ ID NO:3);

CTGACCCTAAGCTCATAAGGATGAAC (SEQ ID NO:4);

CCACCTCATCATCCTGGATGACTTCC (SEQ ID NO:5);

TTAAAAGTGGATTTCATCTTTGTCAGATTCAGG (SEQ ID NO :6);

GGGGACCTCATTTACCATGCTGGAG (SEQ ID NO:7);

GATTCCCTCATCCACATTTTCAAAGGC (SEQ ID NO:8);

and wherein the hybridization reaction is incubated at 42.degree. C. in a solution comprising 50% formamide, 5.times.SSC, and 1% SDS or at 65.degree. C. in a solution comprising 5.times.SSC and 1% SDS, with a wash in 0.2.times.SSC and 0.1% SDS at 65.degree. C.

8. The isolated ***nucleic acid*** of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a homomeric channel.

9. The isolated ***nucleic acid*** of claim 1, wherein the polypeptide monomer comprises an alpha subunit of a heteromeric channel.

10. An expression vector comprising the ***nucleic acid*** of claim 1.

11. A host cell transfected with the vector of claim 10.

12. A method of detecting a ***nucleic acid,*** the method comprising contacting a sample comprising a first ***nucleic acid*** with an isolated second nucleic acid of claim 1 and

detecting hybridization of the second ***nucleic acid*** to the first ***nucleic acid,*** thereby detecting the first ***nucleic acid***.

United States Patent **6,583,337**
Allen , et al. **June 24, 2003**

Plant glucose-6-phosphate translocator

Abstract

This invention relates to an isolated nucleic acid fragment encoding a glucose-6-phosphate/phosphate translocator. The invention also relates to the construction of a chimeric gene encoding all or a portion of the glucose-6-phosphate/phosphate translocator, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the glucose-6-phosphate/phosphate translocator in a transformed host cell.

Inventors: **Allen; Stephen M.** (Wilmington, DE); **Rafalski; J. Antoni** (Wilmington, DE)

Assignee: **E. I. du Pont de Nemours and Company** (Wilmington, DE)

Appl. No.: **436521**

Filed: **November 9, 1999**

Current U.S. Class: **800/278**; 435/6; 435/69.1; 435/71.1; 435/183; 435/410; 435/419; 435/418; 435/252.3; 435/320.1; 530/350; 530/370; 536/23.1; 536/23.2; 536/23.6; 536/24.1; 536/24.3; 536/24.5

Intern'l Class: A01H 003/00; C07H 021/04; C07K 014/415; C12N 005/14; C12N 009/00

Field of Search: 435/6,69.1,71.1,183,410,419,418,252.3,320.1 530/370,350 536/23.1,23.2,23.6,24.1,24.3,24.5

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NCBI General Identifier No. 2997591.
NCBI General Identifier No. 2997589.

Primary Examiner: Bui; Phuong T.

Parent Case Text

This application claims priority benefit to U.S. Provisional Application No. 60/107,910 filed Nov. 10, 1998, now abandoned.

Claims

What is claimed is:

1. An isolated ***polynucleotide*** comprising:

(a) a nucleotide sequence encoding a polypeptide having glucose-6-phosphate/phosphate translocator activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 86% sequence ***identity*** based on the Clustal alignment method, or

(b) the complement of the nucleotide sequence, wherein the complement and the nucleotide sequence contain the same number of nucleotides and are 100% complementary.

2. The ***polynucleotide*** of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 90% sequence ***identity*** based on the Clustal alignment method.

3. The ***polynucleotide*** of claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least 95% sequence ***identity*** based on the Clustal alignment method.

4. The ***polynucleotide*** of claim 1, wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:3.

5. The ***polynucleotide*** of claim 1, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:4.

6. A recombinant DNA construct comprising the ***polynucleotide*** of claim 1 operably linked to a regulatory sequence.

7. A method for transforming a cell comprising transforming a cell with the ***polynucleotide*** of claim 1.

8. A cell comprising the recombinant DNA construct of claim 6.

9. A method for producing a plant comprising transforming a plant cell with the ***polynucleotide*** of claim 1 and regenerating a plant from the transformed plant cell.

10. A plant comprising the recombinant DNA construct of claim 1.

11. A seed comprising the recombinant DNA construct of claim 1.

12. A vector comprising the ***polynucleotide*** of claim 1.

United States Patent **6,582,950**
Smith , et al. **June 24, 2003**

C3 binding polypeptide of Streptococcus agalactiae group b Streptococcus

Abstract

This invention relates to the identification of a human complement C3 binding polypeptide and the nucleic acid which encodes the polypeptide from Streptococcus agalactiae. The polypeptide binds C3 and may be implicated in S. agalactiae adhesion and/or virulence. The polypeptide is conserved in mass in a variety of streptococcal isolates and is recognized by antibodies produced by humans exposed to or colonized with Group B Streptococcus.

Inventors: **Smith; Beverly L.** (Minneapolis, MN); **Ferrieri; Patricia** (Minneapolis, MN)

Assignee: **Regents of the University of Minnesota** (Minneapolis, MN)

Appl. No.: **610199**

Filed: **July 1, 2000**

Current U.S. Class: **435/252.3**; 435/320.1; 435/325; 536/23.7

Intern'l Class: C12N 001/20; C12N 015/00; C12N 005/00; C07H 021/04

Field of Search: 424/190.1 435/69.3,252.33,253.4,252.3,320.1,325 536/23.7

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Foreign Patent Documents

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Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. .sctn.119(e) of U.S. Provisional Patent Application No. 60/157,550, filed on Oct. 4, 1999, and U.S. Provisional Patent Application No. 60/173,766, filed on Dec. 30, 1999, both of which are hereby incorporated by reference.

Claims

What is claimed is:

1. An isolated ***nucleic acid*** fragment that hybridizes to at least a portion of at least one of the ***nucleic acid*** fragments represented by SEQ ID NO:6 or SEQ ID NO:4 or their

complementary strands under hybridization conditions of prehybridization for 1 hour at 62.degree. C. in hybridization solution (5.times.SSC (1.times.SSC is 0.15 M NaCL, 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0.1% N-lauroylsarcosine, 1% Blocking Reagent) followed by two stringency washes with 2.times.SSC, 0.1% SDS for 5 minutes at room temperature and once with 0.5.times.SSC, 0.1% SDS for 15 minutes at 62.degree. C., said isolated ***nucleic acid*** fragment encodes a polypeptide that binds human complement C3 protein.

2. The ***nucleic acid*** fragment of claim 1 isolated from S. agalactiae.

3. The ***nucleic acid*** fragment of claim 1 which encodes a polypeptide represented by. SEQ ID NO:5.

4. The ***nucleic acid*** fragment of claim 1 in a ***nucleic acid*** vector.

5. The ***nucleic acid*** fragment of claim 4 wherein the ***nucleic acid*** vector is an expression vector capable of producing a polypeptide.

6. An isolated ***nucleic acid*** having at least 50% ***nucleic acid identity*** to the ***nucleic acid*** fragments represented by SEQ ID NO:6 or SEQ ID NO:4, and which hybridizes under hybridization conditions of prehybridization for 1 hour at 62.degree. C. in hybridization solution (5.times.SSC (1.times.SSC is 0.15 M NaCL, 0.015 M sodium citrate), 0.02% sodium dodecyl sulfate (SDS), 0. 1% N-lauroylsarcosine, 1% Blocking Reagent) followed by two stringency washes with 2.times.SSC, 0.1% SDS for 5 minutes at room temperature and once with 0.5.times.SSC, 0.1% SDS for 15 minutes at 62.degree. C., to at least a portion of at least one of the ***nucleic acid*** fragments represented by SEQ ID NO:6 or SEQ ID NO:4 or their complementary strands, said ***nucleic acid*** encoding a polypeptide that binds human complement C3 protein.

7. An isolated ***polynucleotide*** encoding a polypeptide comprising the amino acids represented by SEQ ID NO:5.

8. The ***polynucleotide*** of claim 7 wherein the polypeptide binds human complement C3.

9. An isolated host cell comprising a ***nucleic acid*** fragment of claim 1.

10. The cell of claim 9 wherein the cell is a bacterium or a eukaryotic cell.

11. An isolated ***nucleic acid*** fragment comprising SEQ ID NO:6 or SEQ ID NO:4 or their complementary strands.

12. An isolated RNA transcribed from a double-stranded ***nucleic acid*** comprising a ***nucleic acid*** fragment of claim 2.

13. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 50% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

14. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 60% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

15. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 70% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

16. An isolated ***nucleic acid*** fragment encoding a polypeptide having at least 80% amino acid ***identity*** to SEQ ID NO:5, said polypeptide binds to human complement C3 protein.

17. An isolated ***nucleic acid*** fragment consisting essentially of at least 30 nucleotides of SEQ ID NO:4, wherein said ***nucleic acid*** fragment encodes a polypeptide that binds to human complement C3 protein.

United States Patent **6,541,684**
Bowen , et al. **April 1, 2003**

Nucleotide sequences encoding maize RAD51

Abstract

Nucleic acid sequences encoding two RAD51 recombinases active in maize plants are provided. cDNA sequences including the ZmRAD51 coding sequences and unique 3'-untranslated regions which are useful as RFLP probes, are also provided. The production of plasmids containing a nucleic acid sequence encoding a ZmRAD51 fusion protein, as well as the use of the plasmids to introduce the ZmRAD51 coding sequence into a host cell, such as maize cell, are also disclosed.

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Parent Case Text

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/074,745, filed Feb. 13, 1998 and is herein incorporated by reference.

Claims

What is claimed is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:

a) a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 7;

b) a polynucleotide having at least 90% identity to a polynucleotide of (a);

c) a polynucleotide which will hybridize under ***stringent hybridization*** conditions to said polynucleotide of (a) or (b); and

d) a polynucleotide comprising at least 30 contiguous nucleotides from a polynucleotide of (a), (b) or (c);

wherein the polynucleotide of (a), (b) or (c) encodes a polypeptide with recombinase activity.

2. The isolated polynucleotide of claim 1, wherein said polynucleotide has a sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 6.

3. An expression cassette comprising a polynucleotide of claim 1 operably linked to a promoter.

4. The host cell transfected with an expression cassette of claim 3.

5. The host cell of claim 4, wherein said host cell is a bacterial cell.

6. The host cell of claim 4, wherein said host cell is a sorghum or maize cell.

7. A method of making maize recombinase comprising the steps of:

a) transforming or transfecting a host cell with the expression cassette of claim 3; and

b) purifying the recombinase from the host cell.

8. The method of claim 7, wherein the host cell is selected from the group consisting of a bacterial cell, a plant cell, a mammalian cell and a yeast cell.

9. A method of modulating ZmRAD 51 activity in a plant, comprising:

(a) introducing into a plant cell an expression cassette comprising an isolated polynucleotide of claim 1 operatively linked to a promoter;

(b) culturing the plant cell under plant cell growing conditions;

(c) regenerating a plant which possesses the transformed genotype, and

(d) inducing expression of said polynucleotide for a time sufficient to modulate ZmRAD51 activity in said plant.

10. A transgenic plant cell comprising an isolated polynucleotide of claim 1.

11. A transgenic plant comprising an isolated polynucleotide of claim 1.

12. A transgenic seed from the transgenic plant of claim 11.

13. Primer pairs for isolating at least a part of a Zea mays recombinase gene, selected from the group consisting of SEQ ID NOS: 12 and 13, SEQ ID NOS: 14 and 19, SEQ IDS NOS: 14 and 20, and SEQ ID NOS: 14 and 15, or complements thereof.

14. An RFLP probe for a maize recombinase gene comprising at least 30 nucleotides residues of SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11.